MICROSCOPIC EXAMINATION OF BUTTER AND ITS ADULTERATIONS.

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A part of the subject-matter of this paper was published in the winter and spring of this year in Bulletins Nos. 13 and 15 of the Ohio Agricultural Experiment Station. Some of the facts offered for consideration at this time will, therefore, not be new to most members of this Society, but it will be necessary to reproduce them here, in order to made this communication complete in itself as well as to give all of the experimental proof substantiating the conclusions reached.

The papers read by Dr. Thomas Taylor at the last meetings of this Society and the American Association for the Advancement of Science, the publication and general dissemination of an abstract from the Proceedings of the latter society, and the frequent references to it by the agricultural and daily press, the approval of the method by the Department of Agriculture, coupled with the statement, that a number of fraudulent butter dealers had already been convicted on the evidence furnished by the method described, all this coming at a time when the attention of the whole country was otherwise directed to the adulteration of butter, caused almost universal attention to be directed to the study of the new discoveries claimed to have been made.

The microscopic method of Dr. Taylor for the examination of butter and fats may be divided into three parts.

1. The detection of foreign bodies other than fat.

2. The examination of the commercial article by means of polarized light without further treatment.

3. The examination of the crystalline nature of butter and other fats, after boiling, or melting, and gradual cooling, according to directions.

Although the first part of the method seems to contain the only

elements of promise in the microscopic examination of butter for adulterations, from the fact that all butter substitutes are originally prepared in a manner radically differing from the production of genuine butter, and may for this reason contain tissues or other bodies that would distinguish them from genuine butter, yet Dr. Taylor seems to consider it of minor importance. It has not been studied like the other parts of his method, and is merely alluded to as an auxiliary in his investigations. It was omitted entirely from the published abstract mentioned above.

The appliances and materials employed in testing the second and third parts of Dr. Taylor's method were: W. H. Bulloch's biological stand, polarizing apparatus and No. 1 eye-piece; Bausch & Lomb's three-quarter-inch objective, professional series; glass slides, covers, wooden pill-boxes, etc.; pure table butter made at the University dairy; unsalted butter; pure ordinary lard; pure "oleo oil" or tallow fat; a sample of fraudulent butter containing at least ninety-five per cent. of adulteration; a sample of butterine containing twenty-eight per cent. of butter; two samples of genuine creamery butter; olive oil, salt and water.

Part second of Dr. Taylor's method was also omitted from the abstract first published; but since it subsequently appeared in the printed Proceedings of this Society, as well as in the Report of the Department of Agriculture for 1885, and was illustrated by two colored engravings, and since, in an open letter replying to Bulletin No. 13, Dr. Taylor speaks of this as one of his most important tests, it was given a fair trial and the results published in Bulletin No. 15 of the Ohio Agricultural Experiment Station, dated May 10, 1886.

It is but proper to state in this connection, that Prof. J. H. Long, of Chicago, independently and at the same time made a report on this subject to the Illinois State Microscopical Society, in which he arrived at similar conclusions, and offers explanations for the behavior of butter under this test, similar to those given in Bulletin No. 15.

In an open letter, dated March 21, 1886, in describing this test for oleomargarine and butterine, Dr. Taylor says: "Place a green selenite between the polarizer and analyzer, and a plain green color

will be observed. Then place a mounted slide of normal butter under the selenite, view again, and the same even green color is observed. Now place a small portion of lard or beef fat oil on the same slide, and with the polarized light and the same green selenite the fat will exhibit prismatic colors, showing the contrast between a polarizing body such as the semi-solid fats (lard or tallow) and a non-polarizing body like butter."

In accordance with these directions a small quantity of newly churned butter was placed on a glass slide and pressed to a thin film with a covering glass. The polarizer of the microscope having been turned till the green color produced by the selenite appeared, the slide with the butter was placed in position. The result was a green field without prismatic colors.

A sample of alleged creamery butter, which had been sent to the laboratory for examination, and which was found to contain at least 95 per cent. of adulteration, was next subjected to this test under the same conditions. Prismatic colors were plainly visible all over the field of view.

Here the statements of Dr. Taylor are directly confirmed, and it must be conceded that an opinion, based upon this test, would in this case be in accordance with the facts. But, in spite of this coincidence, the conclusions which Dr. Taylor draws from his observations are fallacious and opposed to all law and reason. He ascribes this difference of behavior under the microscope with polarized light to distinctive properties of the various fats; whereas it is strictly due to the difference of conditions in the treatment of these fats. In other words, butter will show these prismatic colors or not, according to the conditions to which it has been subjected, and so will such fats as tallow and lard or their mixtures. Now, if these conditions obtain in the manufacture of the commercial products, or during the time in which they are in the market, it is manifest that the opinion of a person who is called upon to examine a suspected butter, and who is necessarily unacquainted with the history of the article, would be the merest guess-work if based upon this test.

That these conditions do obtain will be seen from the following experiments:

A portion of the butter, which showed the even green color

above, was melted at 104 degrees Fahrenheit. On cooling it was again subjected to the same test, and now it showed the prismatic colors as plainly and strongly as the adulterated butter.

But again, about half a pound of butter, employed in making the experiments in Bulletin No. 13, was left standing in a closed tin box in the laboratory. The butter had become alternately soft and hard as the temperature of the room varied, but had never actually melted. When a slide of this pure butter was submitted to this test it revealed the same display of prismatic colors as the adulterated butter.

Two samples of butter, which had been sent to the laboratory for examination and were found to be unadulterated, were subjected to this test as soon as received. Both samples revealed prismatic colors in a high degree.

On the other hand a mixture of equal parts of oleo oil and lard, which strongly revealed the prismatic colors, was melted at a gentle heat, the melted fat poured into a beaker of cold water and stirred with a glass rod. The fat immediately solidified, forming a hard lump around the stirring rod. When this fat was mounted and examined under the microscope with polarizer and selenite, it showed a uniform green field and no prismatic colors, thus behaving exactly like the pure butter above.

The adulterated butter mentioned above behaved in the same manner when subjected to the treatment just described.

Explanation.—The change in the behavior of butter and other fats under these varying conditions can readily be explained. The reason why butter which has never been exposed to a temperature high enough to soften it shows a green field free from prismatic colors in this test is due, not to the fact, as Dr. Taylor claims, that it is a non-polarizing body, but to the fact that from the nature of its production the fat crystals contained in it are extremely minute. The effect of these minute crystals on the polarized ray is so obscure, that it is completely masked by the green color produced by the selenite. The effect upon the polarized ray produced by melting and cooling butter, or exposing the same to temperatures at which it softens or hardens, is simply due to the fact that the conditions are favorable to the formation of larger fat crystals, in the first place by recrystallization, and in the second place by giving the small crystals an opportunity of growing.

On the other hand, by melting the mixture of tallow and lard as well as the adulterated butter, as was done in the experiments described above, the fat crystals which they contained and which caused the prismatic colors with polarized light and selenite, were destroyed. The instantaneous solidification of these melted fats necessarily produced very minute crystals of fat, and hence, for the same reason as given for fresh butter, their effect upon the polarized ray was not visible.

If it is borne in mind that in the transportation and handling of butter, especially country butter, during the summer months repeated exposure to temperatures high enough to soften butter is very liable to occur, and, on the other hand, that in the manufacture of butterine the melted fats of tallow and lard are run into and churned with cold milk or cold salt water, it will be seen that, so far as this test is concerned, samples of genuine butter would be liable to be condemned, while substances containing little or no butter might be stamped as genuine butter.

The third part of Dr. Taylor's microscopic method of examining butter and fats is the one which has received the most attention, not only because the author has attached the greatest importance to it in his papers and lectures, but because it was alone mentioned in the abstract referred to above. It is asserted in the description of this method that butter can be distinguished from tallow and lard by means of the microscope with polarized light. The claims on which the method is based are as follows:

Pure butter, when treated according to directions given, yields globular crystals, called butter crystals. With polarized light these crystals exhibit a black St. Andrew's cross, and when a selenite is used, in addition, a display of colors with the cross in faint outline. Secondary crystals of rosette forms are also occasionally seen. These forms are never seen in pure beef or lard fats. The globular crystals are visible to the naked eye, and when viewed with a pocket lens appear like insect eggs. Lard, when properly cooled, yields well-defined stellar crystals with or without an opaque center. Beef fat forms crystals with long bi-serrated spines. All of these forms are illustrated by drawings.

It is further claimed, in order to apply the discoveries to the

examination of commercial butter for adulterations, that on boiling and cooling a suspected sample, the butter, lard and tallow, if present, will each be represented by its characteristic crystals.

For the sake of studying the behavior of these fats, and of acquiring an acquaintance with their appearance when treated as described, three preliminary experiments were made at the same time, in which butter, lard and oleo oil were employed respectively.

Experiment 1.—Of the butter made at the University dairy, about half an ounce was transferred to a test-tube, fused in the flame of a Bunsen burner and gently boiled for one minute. It was then poured into a wooden pill-box and allowed to stand until the next day. A small particle of the butter was then taken up on the point of a wooden tooth-pick and mixed on a glass slide with a drop of olive oil. On being stirred in the oil it readily separated into globular bodies visible to the naked eye, and, after being covered with an ordinary covering glass and viewed with a pocket lens, these globular bodies presented the appearance of insect eggs. When viewed under the microscope with transmitted light the magnified globules seemed to consist of a mass of crystalline matter, the individual crystals radiating from a center. When viewed with polarized light most of the globules revealed a well defined black cross. In some of the globules the cross was fainter, and in others distorted, owing, no doubt, to accidental imperfections in the formation of the globules or to injuries in mounting. When a selenite was introduced between the slide and the polarizer, the sections of the globule formed by the cross were alternately colored red and green.

The appearance of these globular bodies is so pronounced under these conditions that, when once seen, a person would have no difficulty in recognizing them again, nor in satisfying himself that they are all similar bodies, even when the cross and distribution of the colors are less perfect (see *figures 1 and 2*).

Experiment 2.—A portion of ordinary pure lard was treated in the same manner as the butter. On account of the absence of water it of course did not boil. The cooled mass was examined the next day. The particle stirred up on a glass slide with olive oil separated with difficulty. When covered and viewed with a pocket lens only minute specks were visible. When examined under the microscope

with polarized light nothing but small, irregular, stellar bodies and masses of isolated fat crystals could be noticed. No globular bodies like those obtained from the butter could be found.

Experiment 3.—A portion of the oleo oil was treated in the same manner as the butter and lard. This substance, like the lard, did not boil, as no water was present in it. The cooled mass was solid and white like a stearin candle, the yellow color of the oleo oil having been destroyed by heating. It separated with difficulty when stirred with olive oil on a glass slide. Neither the pocket lens, nor the microscope with polarized light, revealed any of the globular bodies obtained from butter. Nothing but very minute crystals and stellar forms could be seen.

These three experiments fully corroborate the results obtained by Dr. Taylor in his study of the behavior of butter, lard and beef tallow separately, as described in his paper, except that at this point no particular attention was paid to any difference between the lard and tallow.

If the problem to be solved were to distinguish between butter and lard, or between butter and tallow, then the method would seem to be satisfactory; but it is safe to say, that no analyst or microscopist would ever be called upon to make a decision of this kind. The actual problem is to distinguish between genuine butter and those fraudulent substances which, by mixing, churning, salting and working, are made to resemble the genuine article as nearly as possible in color, taste, grain and consistency. If butter, lard and oleo or tallow, were homogeneous compounds, and further, if the bodies described by Dr Taylor as characteristic of these fats, were crystals, then there could be no reasonable doubt as to the feasibility of obtaining by this process the most positive evidence of the nature of a suspected article of butter. But here is the weakest point in this process. Butter, lard and oleo are not definite chemical compounds. As is well known, they are mixtures of various fats, among which it is necessary to mention stearin, palmitin and margarin. The bodies which Dr. Taylor describes as characteristic for butter, lard and tallow, and which he terms crystals, are not crystals in the true sense of the word, but crystalline aggregates. Crystals are homogeneous bodies bounded by plane surfaces, and

whatever the circumstances under which they occur may be, they are always produced according to fixed laws. Crystalline aggregates may be homogeneous or not, and in their formation they are governed by no law. It is evident from their complex nature that their formation is possible only under the most favorable conditions. If these conditions are modified, the form of the aggregations are modified or their formation prevented entirely. Hence the three experiments already described are not a fair test of the process; although singularly enough it has been claimed by some writers, since the publication of Bulletin No. 13, that these three tests were the only ones made which had any relation to Dr. Taylor's method. It is claimed, as already mentioned, that by this method tallow fat can be detected in butter adulterated with it. To test this claim the following three experiments were made, and they were surely in order.

Experiment 4.—A mixture consisting of 90 per cent. butter and 10 per cent. oleo was boiled and cooled as in the experiment with butter. The mounted slides could not be distinguished from the pure butter slides.

Experiment 5.—A mixture was next made of 75 per cent. butter and 25 per cent. oleo, and treated as before. Again, no difference could be noticed between this adulterated butter and the pure butter. The globular bodies were large and perfect, the cross as distinct as in the pure butter, while the slides were remarkably free from any small crystals and bodies which might be taken for the adulteration.

Experiment 6.—Equal parts of butter and oleo were next mixed together and subjected to the same treatment. The results, after a most careful examination, were essentially the same as in the preceding experiment. Assuming that the tallow fat, with which the pure butter was adulterated in the last three experiments, crystallizes out by itself in the form described by Dr. Taylor, or in the form exhibited in experiment three, it would be possible to overlook the tallow fat "crystals," when the adulteration is only 10 per cent. This oversight is less probable in the next experiment with 25 per cent. of adulteration, and is entirely out of the question when, as in the last experiment, the adulteration amounts to about 50 per cent.

Hence it is manifest, that in these three experiments the tallow fats united with the butter fats in forming the globular bodies. These results are just as they should be according to the theoretical consideration given above. After boiling, the mixture of butter and oleo is a homogeneous one. When the temperature has fallen to the point at which the stearin begins to crystallize, it would not only be unreasonable, but it would be absolutely absurd to claim, that the molecules of stearin introduced into the mixture by the tallow fat should keep aloof from the molecules of the stearin of the butter, and form little crystals and crystalline aggregates all by themselves. On the contrary, in the present light of science, it is safe to maintain that every individual crystal of stearin formed from the mixtures described above contains molecules from each source just in the relative proportion of stearin present in the ingredients forming the mixtures.

Experiment 7.—The difference in behavior of the tallow fats in experiment three and the last three experiments can only be ascribed to a difference of conditions. It is well known that table butter normally contains 4 to 6 per cent. of salt and 5 to 20 per cent. of water. In order to test the effect of this admixture upon the tallow fats, about half an ounce of the oleo oil used in experiment three was mixed in a porcelain mortar with a small quantity of salt and eight or ten drops of water. After the water was thoroughly incorporated, the mass was transferred to a test-tube, and boiled for one minute, as in the case of butter. It was then poured into a wooden pill-box and allowed to cool as before. The cooled mass presented quite a marked difference in appearance from that obtained from the same substance in experiment three. It retained to a great extent the yellow color of the oleo, was of a more granular nature, and in fact resembled boiled butter in every respect. When a small particle was stirred up with olive oil on a glass slide it separated readily. When covered and viewed with a pocket lens it revealed a mass of globules resembling insect eggs. Under the microscope the globules exhibited essentially the same characteristics as those obtained from pure butter. The individual crystals forming the oleo globules seemed coarser and fewer in number, and to this circumstance was ascribed the fact that the cross as

well as the colors produced by the selenite were less sharply defined than in most of the butter globules thus far observed. In a repetition of this experiment made from another portion of the same oleo a few weeks since, the globules showed the cross as perfectly as in the case of butter. Hence there can be no doubt that the globular bodies obtained from the oleo are in every way similar to those obtained from butter. (See *fig. 5*, Plate VI.)

Experiment 8.—Having thus discovered that these globular bodies can be obtained from pure tallow fat by boiling and cooling, after adding the minimum of salt and water normally present in table butter, the following test was made: Nine grams of oleo and one gram of lard were put into a small beaker, and eight or ten drops of a saturated solution of salt in water added. The mixture was then gently heated to melt the fats. After shaking violently for a a few moments to mix the salt solution with the fats, the mixture was boiled gently for one minute and then allowed to cool in a wooden pill-box as before. The microscopic examination revealed globular bodies, which could in no wise be distinguished from those obtained from pure butter. The crystalline texture was dense, the cross plainly marked, and the colors produced by the selenite sharply defined.

Experiment 9.—A mixture of one part of lard and five of oleo was treated in the same manner as in the last experiment, with like results.

Experiment 10.—A similar test was made with a mixture containg 80 per cent. oleo and 20 per cent. lard. In this case the individual globular bodies were very large and perfect, owing no doubt, to favorable conditions during the time of crystallization. (See *fig. 3*, Plate VI.)

Experiment 11.—From a mixture of equal parts of oleo and lard under the same treatment as above, perfect specimens of the globular bodies were obtained. They were, however, comparatively small, and were surrounded by small, detached crystals of fat. The occurrence of isolated fat crystals in this case may have been due to the excess of olein added with the lard, but more probably it was brought about by too rapid solidification of the mass after the temperature had fallen to the crystallizing point, as this would at the

same time account for the smaller size of the globular bodies present.

The methods employed in obtaining the characteristic "butter crystals" from oleo and mixtures of oleo and lard have been criticised by various writers since the publication of Bulletin No. 13.

In the first place, objections were raised to employing salt and water in making the experiments. The only reason for adding salt and water was to bring the fats in this respect under the same conditions which obtain in table butter, the nature of which the author of the method claims to be able to determine. A simple calculation, from the data given in the description of the experiments, will show that not more than 4 or 5 per cent. of water was used, which is the minimum found in commercial butters, pure and fraudulent. This objection is not well founded.

Again, it was claimed that an error was committed in boiling the mixtures and then allowing them to cool. Now, in view of the fact that the author of this method has stated over and over again that his "butter crystals" are produced only by boiling and subsequent cooling, it is difficult to conceive why this objection should have been raised.

But in order to remove the cause of the first objection, two further tests were made, employing the fraudulent butters mentioned in the beginning of this paper. One of these butters contained at least 95 per cent. and the other 68 per cent. of foreign fats. They were subjected to the test just as they were obtained in the market. Both specimens yielded excellent "butter crystals," and practically nothing but "butter crystals" could be found. Both samples, according to this test, would have been declared genuine butter. (See *fig. 6*, Plate VI.)

CONCLUSION.

The only conclusion which can be drawn from the experiments which have just been described, and which were made conscientiously and without prejudice, is that the microscopic methods as laid down by Dr. Taylor are of no practical value in the examination of butter for adulterations. This conclusion, however, although it follows from what has gone before, leaves one important question untouched, and that is, Why should different observers, who are working honestly for the same end, under the same instructions, and

with the same material, arrive at such discordant results as have characterized a great many of the observations which have been made on this subject within the last year? The answer is simply the fact already stated, that the bodies to which attention has been directed are not true crystals formed according to unchangeable laws, but are complex aggregates of crystals, which vary in characteristics and form as the conditions vary under which they are produced. Observers are liable to ignore slight modifications of conditions and to ascribe certain results thus produced to the material in hand.

An experiment made a few weeks ago shows how a variation of temperature will affect results. About an ounce of a mixture of 80 per cent. oleo and 20 per cent. lard was mixed with a little salt water and boiled for one minute. Two wooden pill-boxes were filled with the hot fat. One box was placed on a small piece of ice which was wrapped in paper, while the other box was exposed to ordinary temperature of the room. On cooling, this box contained the most perfect "butter crystals" (see *fig. 4*, Plate VI.), while the box, which had been more rapidly cooled near the ice, contained no "butter crystals" at all, but that form described by Dr. Taylor as the beef and fat crystal. No two preparations could look more unlike, yet they were poured from the same beaker at the same time, and merely allowed to solidify at different temperatures. The difference of temperature to which these two boxes were exposed is not greater than the variation of temperature in rooms during the colder season. One person may be working in a room heated to a temperature of seventy-five or eighty degrees, while another might, if busy at work, unconsciously let the temperature of his room fall to fifty or forty degrees. It can readily be seen, from the illustrations just given, that these two persons working with the same material would obtain conflicting results. If in addition to this the experimenter in the cold room should use a smaller quantity of material in his operation, his results would be still further removed from those of the other experimenter, because a smaller quantity of material will solidify in a shorter time than a larger quantity.

To the same modifications of conditions, although more slight, and it may be said so slight as to be beyond the control of the

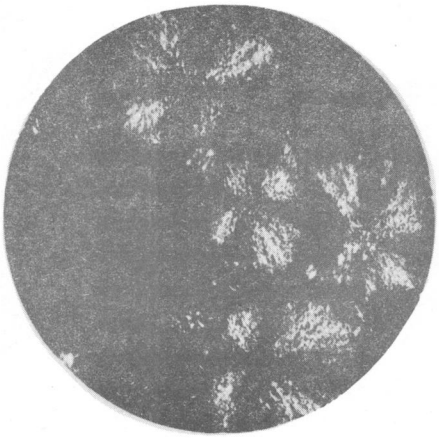

FIG. 1.

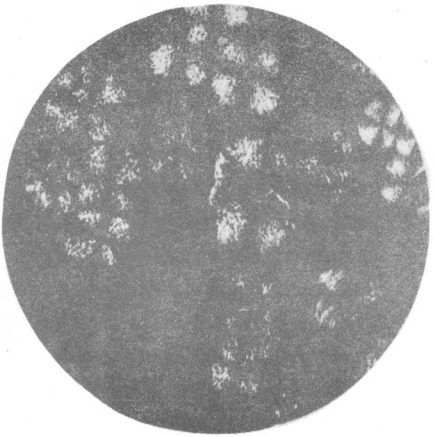

FIG. 2.

FIG 3.

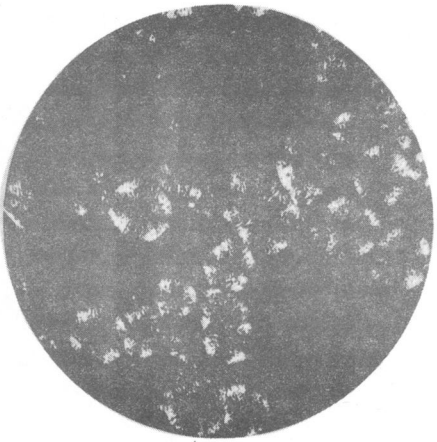

FIG. 4.

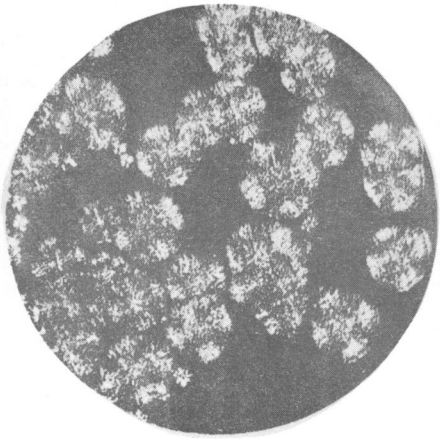

FIG. 5.

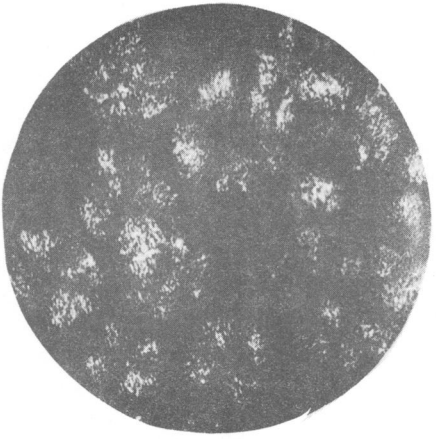

FIG. 6.

EXPLANATION OF PLATE VI.

The figures of Plate VI. were prepared from photographs made with polarized light by Dr. H. J. Detmers, Professor of Veterinary Surgery, Ohio State University, Columbus, Ohio.

FIG. 1.—Pure butter.

FIG. 2.—Pure butter.

FIG. 3 —Eighty per cent. oleo and twenty per cent. lard.

FIG. 4.—Eighty per cent. oleo and twenty per cent. lard.

FIG. 5.—Pure oleo.

FIG. 6.—Commercial butterine, containing ninety-five per cent. of adulteration.

experimenter, must be ascribed such delicate variations in appearance of the "butter crystals," which Dr. Taylor considers to be due to dry feeding and grass feeding of the animals, or to different breeds of cattle, or even to localities from which the butter was obtained. The indented "butter crystal," which Dr. Taylor has figured and described, can be accounted for much more rationally than to ascribe it to Tennessee butter made from shorthorn Devonshire cows, and at the same time experimental proof can easily be obtained to substantiate the explanation. These globular bodies consist of elongated, scaly or acicular fat crystals, radiating in all directions from a center. It is evident that, owing to the divergence of these crystals, the outer part is less firm than the inner, and also that the outer part of the larger globules is less firm than that of the smaller ones in the same preparation. The indentations are produced merely by the pressure of the firmer, smaller globules into the softer and larger ones, when they are massed together. If in mounting a slide the material be taken from the bottom of the box instead, as is usually done, from the top, the indented "butter crystals" will be found, and it matters not whether they are made from Ohio or Tennessee butter, from oleo, or from mixtures of oleo and lard.